JOURNAL OF VIROLOGY, Sept. 1994, p. 6038–6046
0022-538X/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 68, No. 9

Identification of an *H-2 K^b*-Presented Moloney Murine Leukemia Virus Cytotoxic T-Lymphocyte Epitope That Displays Enhanced Recognition in *H-2 D^b* Mutant bm13 Mice

ALICE J. A. M. SIJTS, MARLOES L. H. DE BRUIJN, MAAIKE E. RESSING, JOHN D. NIELAND,† ERICA A. M. MENGEDÉ, CLAIRE J. P. BOOG,‡ FERRY OSSENDORP, W. MARTIN KAST, AND CORNELIS J. M. MELIEF*

Department of Immunohematology and Blood Bank, University Hospital Leiden, 2300RC Leiden, The Netherlands

Received 18 January 1994/Accepted 9 June 1994

Upon infection with the Moloney murine sarcoma virus-murine leukemia virus (MuLV) complex, *H-2^b* C57BL/6 (B6) mice respond with a class I *D^b*-restricted cytotoxic T-lymphocyte (CTL) response, which protects against virus-induced tumorigenesis. In the B6-derived *D^b* mutant B6.CH-2^{bm13} (bm13) strain, part of the class I *D^b* antigen-presenting groove is shaped by a class I *K^b*-encoded sequence. Like B6 mice, bm13 mice reject Moloney virus-induced tumors, but the protective CTL response is *K^b* restricted. In this study we show enhanced levels of Moloney MuLV-specific CTLp with a restriction for *K^b* in bm13 mice. Through the use of CTL clones from Moloney virus-immunized bm13 mice, the class I *K^b*-presented CTL epitope was identified. The epitope is located in the Moloney virus gp70 envelope protein region {Moloney envelope, amino acids 189 to 196 [Mol env (189-196)]}, SSWDFITV and has the *K^b* allele-specific binding motif. The *D^{bm13}* molecule does not present the env(189 to 196) epitope to *K^b*-restricted bm13 CTL. In B6 mice, Mol env(189-196)-specific CTL could be induced by peptide vaccination. B6 mice thus have CTL precursors specific for this epitope but at considerably lower levels than do bm13 mice. We hypothesize that additional positive selection of *K^b*-restricted CTL on the *D^{bm13}* molecule in bm13 mice explains this difference in precursor frequencies. We examined related strains of MuLV for the presence of Mol env(189-196) sequence equivalents. Rauscher, Friend, and AKV MuLV-encoded Mol env(189-196) epitope equivalents were properly recognized in cytotoxicity assays, both as synthetic and as endogenously expressed (Rauscher MuLV) peptides. In contrast, the mink cell focus-forming virus MuLV-encoded epitope equivalent, lacking a *K^b* anchor residue, was not presented for CTL recognition and hence can be excluded as an important CTL epitope for mink cell focus-forming viruses.

Injection of C57BL *H-2^b* mice with the Moloney murine sarcoma virus-murine leukemia virus (MSV-MuLV) complex leads to rapid tumor development at the site of virus inoculation. Tumors spontaneously regress within 6 weeks, largely because of cytotoxic T-lymphocyte (CTL) activity (21). This CTL response is directed against the tumor virus MuLV component and is mainly restricted by *H-2* class I *D^b* in B6 mice (*H-2* class I *K^b*, *D^b*) (6, 14, 21, 41). B6.C-H-2^{bm13} (bm13) mice are genetically identical to B6 mice, except for a *K^b* gene-derived nucleotide sequence within the class I *D^b* locus. This results in three amino acid substitutions within the β-pleated sheet of the antigen-presenting groove of the bm13 *D^b* molecule (*D^{bm13}*), which consequently over a stretch of 13 amino acids (aa) (positions 108 to 119) resembles the antigen-presenting groove of class I *K^b* (11). Interestingly, bm13 and B6 mice show the same pattern of tumor development and regression upon infection with Moloney virus, but CTL reactivity in bm13 mice is class I *K^b* restricted (38, 39, 40). *D^b* mutant B6.C-H-2^{bm14} mice, like bm13 mice, lack class I *D^b* (*$D^b \rightarrow D^{bm14}$*), but despite also bearing class I *K^b*, they do not generate detectable Moloney virus-directed CTL reactivity upon infection (40).

CTLs recognize short peptides which are presented in the antigen-binding groove of major histocompatibility complex (MHC) class I molecules (2, 31, 43, 44). This serves a dual function. First, constitutively presented self peptides determine the selection of the CTL repertoire during immune development (1, 15, 24, 29, 34), and second, the specific recognition of class I-presented antigenic peptides by mature CTLs induces responsiveness (31, 43, 44). The peptides presented on cell surface-expressed class I molecules evolve from endogenous processing of cytosolic proteins (23). Assembly of major histocompatibility complex class I-peptide complexes presumably takes place in the rough endoplasmic reticulum and occurs in an allele-specific fashion. The rules for peptide binding are defined by the structure of the antigen-binding groove of the class I molecule (5, 33, 45). Peptides binding to *H-2* class I *K^b* are characterized by a length of 8 to 9 aa and carry a Phe (F) or Tyr (Y) residue at position 5 and an aliphatic residue at the carboxy peptide terminus (the so-called anchor residues for peptide binding). Specific mutations in the class I antigen-binding groove, as in the *D^b* mutant *D^{bm13}* or *K^b* mutant *K^{bm1}*, alter the repertoire of peptides being bound (30, 45), and thereby the CTL repertoire.

To investigate in which way the *D^{bm13}* molecule influences the repertoire of Moloney MuLV-specific CTL in bm13 mice, we identified the immunodominant *K^b*-restricted CTL epitope, expressed on Moloney MuLV-infected bm13 cells. We used a set of viral peptides with the *K^b*-allele-specific motif that strongly bound this class I molecule. The peptides were derived

* Corresponding author. Mailing address: Department of Immunohematology and Blood Bank, University Hospital, Building 1, E3-Q, P.O. Box 9600, NL-2300RC Leiden, The Netherlands. Fax: 31 71 21 67 51.

† Present address: Division of Immunology, The Netherlands Cancer Institute, 1066CX Amsterdam, The Netherlands.

‡ Present address: Department of Immunology, Veterinary Institute, 3508 TD Utrecht, The Netherlands.

from the viral core (gag) and envelope (env) proteins. By gene transfection both of these proteins have been shown to encode epitopes for MuLV-specific CTL in $H\text{-}2^b$ mice (6, 14). In addition, for Friend MuLV an env-encoded 17-mer peptide, encompassing a class I D^b-presented CTL epitope, has been described (32), and we recently identified a K^b-presented env-encoded CTL epitope for mink cell focus-forming virus (MCF) MuLV-specific CTL (37). In the present study, we found the immunodominant Moloney epitope for bm13 CTL to be encoded in the MuLV gp70-env region, aa 189 to 196 [Mol env(189-196)], amino acid sequence SSWDFITV. The peptide is presented by class I K^b but not by class I D^b or D^{bm13}.

Cross-reactivity of MuLV-specific CTL toward related viruses is generally observed (9, 12, 28). We investigated the fine virus specificity of Mol env(189-196)-directed CTL and found that Rauscher, Friend, and AKV MuLV encode an epitope equivalent which is recognized by env(189-196)-specific CTL and is processed in Rauscher MuLV-transformed tumor cells. In contrast, the MCF MuLV-encoded epitope equivalent lacks the position 5 anchor residue for K^b binding, poorly binds the K^b molecule, and is not presented on MCF MuLV-transformed tumor cells.

MATERIALS AND METHODS

Mice. Mice used in this study were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands, and were held under pathogen-free conditions. Haplotypes: C57BL/6 (B6), $H\text{-}2K^b$, D^b; B10.A(4R), $H\text{-}2K^k$, D^b; B10.A(5R), $H\text{-}2K^b$, D^d. B6.CH-2^{bm13} (bm13), $H\text{-}2\ K^b$, D^{bm13} is a spontaneous B6-derived D^b mutant strain (11), carrying the bm13-specific mutations in the D^b molecule: position 114, Leu→Gln, position 116, Phe→Tyr and position 119, Glu→Asp.

Cell lines. RMA and its derivate RMA-S are Rauscher MuLV-transformed T-cell lines ($H\text{-}2^b$) (22), EL4 is a carcinogen-induced thymoma cell line ($H\text{-}2^b$) (10). 717 is an MCF1233 MuLV-transformed T-cell line and 771 is an MCF1233 MuLV-transformed B-cell line (37); both are derived from MCF1233 MuLV-infected C57BL/10 mice as described previously (47). FRE (Fisher rat embryo) cells and the FRE class I transfectants FRE.B2 (class I D^b), FRE.R3 (class I K^b) and FRE.V4 (class I D^{bm13}) (14) were a gift of F. Lilly (Albert Einstein College of Medicine, Bronx, N.Y.). All cell lines were maintained in Iscove's medium (Seromed; Biochrom KG, Berlin, Germany) with 5% fetal calf serum. FRE class I transfectants were incubated with gamma interferon (10 U/ml; Cetus) for 48 h prior to use in CTL assays.

Generation of Moloney virus-specific CTL. In mice, sarcomas were induced by intramuscular inoculation of 100 μl (9×10^2 focus-forming units, determined on SC-1 mouse cells) of Moloney MSV-MuLV (27) as described previously (41). After complete tumor regression, spleens were taken out and brought into suspension. Spleen bulk cultures were restimulated in vitro with irradiated Moloney MuLV-infected syngeneic lipopolysaccharide (LPS)-stimulated blasts (41) once a week. The Moloney MuLV-specific CTL clones were derived from a limiting dilution of Moloney MuLV-specific bm13 CTL bulks as described previously (16). Culture was in Iscove's modified Dulbecco medium (Gibco BRL) supplemented with 10% fetal calf serum, penicillin (100 IU/ml), kanamycin (100 μg/ml), L-glutamine (2 mM), 2-mercaptoethanol (20 μM), and culture supernatant from phorbol myristate acetate-concanavalin A-stimulated rat spleen cells (17) at 37°C in humidified air containing 5% CO_2.

CTL precursor frequency analysis. Twenty-four replicate microcultures with various numbers of responder spleen cells (spleen cells from mice that underwent complete tumor regression after infection with Moloney virus) were stimulated with 5×10^4 irradiated syngeneic Moloney MuLV-infected LPS-stimulated blasts (41) and were cultured in 200 μl of Iscove's culture medium, supplemented with rat concanavalin A supernatant, in U-bottom tissue culture plates (Costar, Cambridge, Mass.). At day 7, the cytolytic activity of 100 μl of cell suspension in each well was tested in a 4-h ^{51}Cr release assay (see below), using Moloney MuLV-infected LPS-stimulated blasts (41) as the target (5×10^3 per test well). Positive cultures were defined as those in which the ^{51}Cr release values exceeded by 3 standard deviations the spontaneous release values. Minimal estimates of CTL precursor (CTLp) frequencies were calculated from the zero-order term of the Poisson distribution with linear regression analysis by the least-squares method (42).

Peptides. Eleven-mer peptides of Moloney MuLV gag and env proteins were synthesized in small amounts by the Pepscan method (46) at the Central Veterinary Institute, Lelystad, The Netherlands. Pepscan peptides were dissolved in 500 μl of phosphate buffered saline (PBS) and were stored at −80°C. Peptides, required in bulk amounts, were synthesized on a multiple peptide synthesizer (Abimed AMS 422) (8) at the Department of Immunohematology and Blood Bank, University Hospital Leiden, Leiden, The Netherlands. These peptides were analyzed for purity by reverse-phase high performance liquid chromatography, lyophilized and dissolved in PBS. Stock solutions of 5 mg/ml were stored at −80°C.

Generation of peptide-specific CTL. B6 mice were immunized with 100 μg of peptide in incomplete Freund's adjuvant (1:1) in the base of the tail. At day 9 spleens were taken out, brought into suspension, and passed over nylon wool. RMA-S cells were preincubated for 48 h at 26°C in medium with 2% pooled human serum and were subsequently loaded with peptide (100 μg/ml) under serum-free conditions (4 h at 26°C). After mitomycin C treatment (50 μg/ml, 1 h at 37°C) and irradiation (2,500 rads), the peptide-loaded RMA-S cells were washed three times and used to restimulate the syngeneic nylon wool-passed spleen cells (R/S ratio, 4:1). Culture was performed in 96-well U-bottom plates (Costar) in Iscove's Gibco medium, supplemented as described above. Culture supernatant from phorbol myristate acetate-concanavalin A-stimulated rat spleen cells (17) was added after 1 week of culture. Cells were restimulated at day 8 with peptide-loaded irradiated B6 spleen cells, and no free peptide was added to the CTL cultures.

CTL assays. CTL assays were performed as described previously (4). In short, 1×10^3 to 2×10^3 $Na_2{}^{51}[Cr]O_4$-labeled target cells were added to various numbers of effector cells and were incubated for 5 to 6 h at 37°C. The percentage of specific ^{51}Cr release was calculated as ([cpm experimental release] − [cpm spontaneous release])/([cpm 1% Triton X-100 release] − [cpm spontaneous release]). All assays were carried out in triplicate. For target cell sensitization, ^{51}Cr-labeled target cells were incubated with peptide for 10 min prior to addition to the test wells. Peptides remained present during the assay (concentration, 0.5 μg/ml unless stated otherwise).

***H-2* class I peptide binding assay.** Peptide binding assays were performed as described previously (18), with slight modifications. In short, RMA-S cells were washed twice in serum-free medium and were incubated in medium with 2% human serum instead of fetal calf serum for 48 h at 26°C. Afterwards, cells were washed and seeded in U-bottom 96-well plates, and 2×10^5 cells were incubated with peptide (at concentrations ranging from 0.2 ng/ml to 100 μg/ml) in serum-free medium in

TABLE 1. CTLp frequencies of Moloney virus-specific CTL in B6 and bm13 mice

Responder mouse strain (main restriction)	No. of target cells[a] for haplotype:			
	B6 K^b D^b	bm13 K^b D^{bm13}	B10.4R K^k D^b	B10.5R K^b D^d
B6 (D^b)	1/5,000 ± 2,000	1/160,000 ± 30,000	1/9,000 ± 3,000	1/119,000 ± 20,000
bm13 (K^b)	1/13,000 ± 2,000	1/7,000 ± 1,000	1/70,000 ± 8,000	1/13,000 ± 2,000

[a] Mean minimal estimate of CTLp frequencies of five experiments ± standard deviation. Target cells were Moloney MuLV-infected, LPS-stimulated blasts.

a total volume of 60 μl for 4 h at 37°C. Cells were washed and stained for class I K^b expression as described below.

Immunofluorescence. A total of 2×10^5 cells were incubated with an excess amount of first antibody (B8.3.24 for detection of class I K^b expression) in a volume of 25 μl for 30 min at 4°C. Cells were washed once with PBS containing 0.5% bovine serum albumin and 0.02% sodium azide (PBA) and were subsequently incubated with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Boehringer, Mannheim, Germany) second antibody for 30 min at 4°C. After incubation, cells were washed and suspended in 100 μl of PBA, and fluorescence was measured on a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). The results represent the mean fluorescence intensity of 3,000 gated events. Fluorescence indexes were calculated as follows: ([mean fluorescence in the presence of peptide] − [mean fluorescence in the absence of peptide])/mean fluorescence in the absence of peptide.

RESULTS

Bm13 mice display enhanced levels of Moloney MuLV-specific K^b-restricted CTLp. B6 and B6 D^b-mutant bm13 mice, intramuscularly injected with the Moloney MSV-MuLV virus complex, develop sarcomas within 2 weeks. Tumors regress spontaneously within the following 6 weeks (data not shown). Previously, in bulk cultures it has been shown that bm13 mutant mice (K^b, D^{bm13}) have elevated levels of K^b-restricted Moloney MuLV-specific CTL compared with normal B6 mice (K^b, D^b), in which the CTL response is D^b restricted (38–41). We extended this analysis to the clonal level. Limiting dilution with spleen cells from B6 and bm13 tumor regressor mice showed that the levels of Moloney MuLV-specific precursors are comparable for both haplotypes, 1/5,000 for B6 and 1/7,000 for bm13 when they were tested against syngeneic Moloney MuLV-infected targets (Table 1). In B6 mice this frequency was comparable to that measured against virus-infected 4R target cells (K^k, D^b), confirming D^b restriction. Only a few precursors could be detected against virus-infected bm13 (K^b, D^{bm13}) and 5R (K^b, D^d) target cells (K^b-restricted CTL; <1/100,000). In bm13 mice, apart from the high responsiveness against syngeneic virus-infected cells, similar high precursor frequencies were shown against virus-infected B6 and 5R target cells but not 4R target cells (Table 1), which demonstrates K^b restriction of bm13-derived CTL. These data therefore support the previous observations, showing a discrepancy between the levels of K^b-restricted Moloney MuLV-specific CTLp in bm13 (approximately 1/7,000) and B6 (<1/100,000) mice.

Mapping of the Moloney MuLV-encoded epitope for CTL derived from bm13 mice. Stable CTL lines and clones were established by cocultivation of spleen cells from bm13 mice with complete tumor regression and irradiated syngeneic Moloney MuLV-infected, LPS-stimulated blasts (41). As shown in Fig. 1 for a CTL line and the 10B6 CTL clone, these cultures specifically recognized Moloney MuLV-infected FRE cells transfected with the mouse class I K^b molecule. FRE transfectants carrying mouse class I D^b or D^{bm13} and both untransfected and uninfected FRE cells were not recognized, confirming K^b restriction and Moloney MuLV specificity.

To determine the fine peptide specificity of the CTL cultures, the Moloney-MuLV sequence (35) was examined for peptides that could bind the class I K^b molecule and, thereby, might serve as a CTL epitope. Overlapping peptides (11 aa long, with an overlap of 9 aa) covering the Moloney MuLV gag and env proteins were tested for the ability to upregulate the class I K^b expression on RMA-S cells. This assay is based upon the fact that, because of an intrinsic antigen-processing deficit, RMA-S cells have a low cell surface expression of largely unstable *H-2* class I molecules (22). The stable conformation can be acquired by binding of exogenously added peptides. This results in an elevated class I expression level and is detectable by immunofluorescence (18).

Incubation of RMA-S cells with the 11-mer peptides led to identification of one *gag*- and four *env*-encoded sequences that enhanced the class I K^b expression (Table 2) and contained the described binding motif for the K^b molecule (5). In addition,

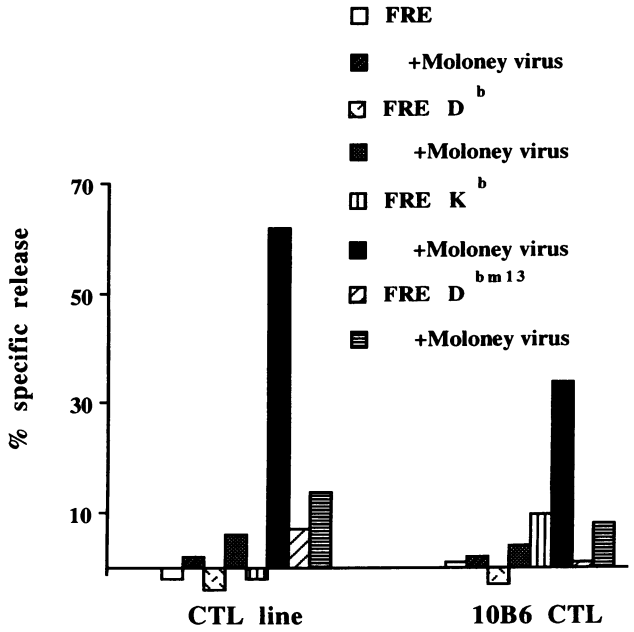


FIG. 1. Lytic activity of a Moloney MuLV-specific bm13 CTL line and CTL clone (10B6) against Moloney virus-infected versus uninfected FRE cells, transfected with class I D^b, K^b, or D^{bm13}. Moloney virus infection and class I expression were checked by FACS analysis (data not shown). The ^{51}Cr release values shown represent means of two triplicate measurements at an effector-to-target (E/T) ratio of 4.

TABLE 2. Recognition of K^b-presented peptides by Moloney virus-specific bm13 CTL[a]

Effector	Peptide[b]	% ^{51}Cr release[c] ± SD
CTL clone 10B6	None[d]	−2 ± 2
	PQSSLYPAL gag(126-134)	−2 ± 2
	SSWDFITV env(189-196)	54 ± 4
	LVDGAYQAL env(317-325)	−2 ± 3
	SPSYVYGLF env(453-461)	1 ± 2
	NRSPWFTTL env(602-610)	−3 ± 3
	NLTTDYCVL env(434-442)	2 ± 5
	MATQQFQQL env(500-508)	2 ± 3
	STQGWFEGL env(592-600)	−1 ± 7
	VLTQQYHQL env(650-658)	−5 ± 4
CTL line	None[d]	6 ± 1
	SSWDFITV env(189-196)	50 ± 1

[a] CTL were added at an E/T ratio of 8.
[b] All peptides are encoded by the Moloney MuLV *gag* or *env* region. Peptides 1 to 5 were selected on the basis of class I K^b stabilization in the RMA-S-binding assay, performed with gag and env 11-mer peptides. Peptides 6 to 9 were selected on the basis of class I K^b-binding motif, and they strongly upregulate the K^b expression on RMA-S cells. Peptides were tested for sensitization of MuLV-negative EL4 target cells, which were preincubated with peptide 30 min prior to CTL addition. Peptide concentration during the assays was 10 μg/ml.
[c] Mean of a triplicate measurement. Similar results were obtained in two independent experiments.
[d] Percent lysis of EL4 cells without peptide.

another four *env*-encoded peptides were selected for the K^b motif (Table 2). All peptides were synthesized as 8- or 9-mer sequences, checked for their ability to upregulate the class I K^b expression on RMA-S, and tested in cytotoxicity experiments. As demonstrated in Table 2, the env(189-196) peptide (SSWDFITV) was capable of sensitizing the Moloney MuLV-negative EL4 target cell line for lysis by the 10B6 CTL clone. The other eight peptides were not recognized in these assays. The

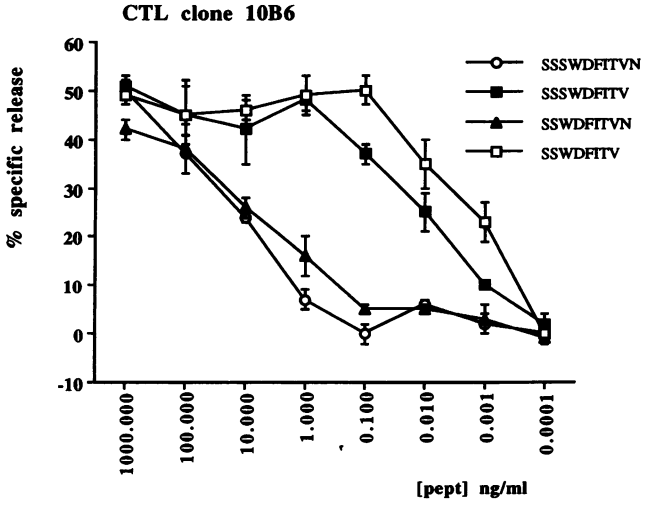


FIG. 2. Sensitization of EL4 target cells (MuLV negative) by length variants of the Moloney MuLV CTL env(189-196) epitope for recognition by the CTL clone 10B6 (E/T ratio, 5). Target cells were added to the titrated peptides (ranging from 10^{-4} to 10^3 ng/ml) 10 min prior to CTL addition. Half-maximal lysis (~25% ^{51}Cr release) was at 0.001 ng/ml for the SSWDFITV octapeptide, at 0.01 ng/ml for the SSSWDFITV nonapeptide, and at 10 ng/ml for the SSWDFITVN nonapeptide and SSSWDFITVN decapeptide.

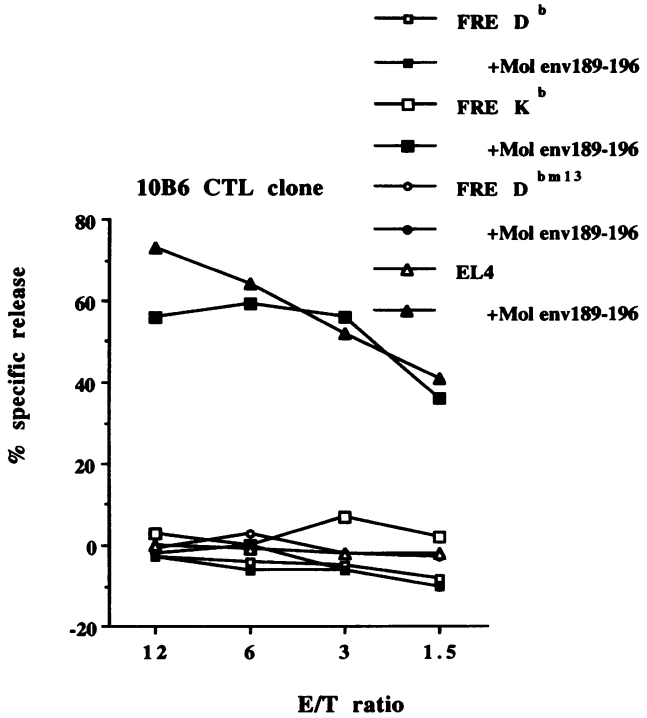


FIG. 3. Lytic activity of the 10B6 CTL clone against class I D^b-, K^b-, or D^{bm13}-transfected FRE cells and EL4 cells with or without Moloney env(189-196) peptide (0.5 μg/ml). Values shown are means of triplicate measurements. Standard deviations were less than 5%. FRE D^{bm13} cells were lysed specifically by D^{bm13}-allospecific B6 CTL in the same experiment (data not shown) and, for all FRE transfectants, class I expression was checked by FACS analysis (data not shown).

Moloney MuLV-specific CTL line also recognized the env(189-196) epitope (Table 2).

Characterization of the Mol env(189-196) CTL epitope. The env(189-196) sequence is encoded in the extracellular region of the Moloney MuLV gp70 envelope cell surface protein. Next to the CTL line and the 10B6 CTL clone described above, we found the env(189-196) peptide to be recognized by two independent Moloney MuLV-specific bulk CTL cultures of bm13 mice and, in addition, by all 40 CTL clones isolated from one of these CTL cultures (data not shown). Possible alternative peptide specificities, as might appear from specific lysis of Moloney MuLV-infected cells but not Mol env(189-196)-loaded cells, were never observed. We therefore conclude that Mol env(189-196) most likely serves as an immunodominant epitope in the Moloney MuLV-directed CTL response in bm13 mice.

In general, class I K^b-restricted epitopes are 8-mer sequences, but longer epitopes (9 or 10 aa long) have been described as well (5). We tested the env(189-196) peptide as well as two 9-mer peptides and a 10-mer one covering this sequence, in a titration on EL4 target cells (Fig. 2). At a high peptide concentration (1 μg/ml) all peptides gave the same level of specific lysis (approximately 50%) when the 10B6 CTL clone was used as effector. The half-maximal lysis level was reached for the env(189-196) octapeptide at 0.001 ng/ml. A 10-fold amount of env(188-196) nonapeptide and a 10,000-fold amount of env(189-197) nonapeptide or env(188-197) decapeptide were required for half-maximal lysis by 10B6 CTL (Fig. 2). Thus, as expected on the basis of the K^b binding motif

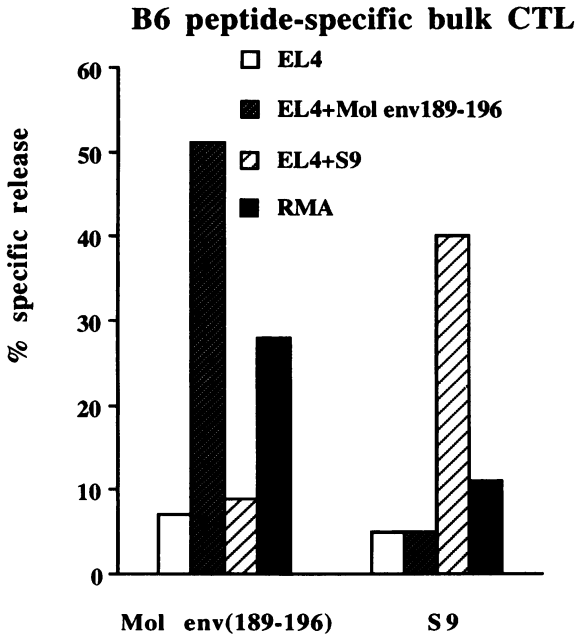


FIG. 4. Lytic activity of day 16 spleen cell bulk cultures (≥80% CD8 positive) of Mol env(189-196) peptide-immunized B6 mice (left four bars) and Sendai virus S9 peptide-immunized B6 mice (right four bars) against EL4 target cells without peptide or loaded with Mol env(189-196) or Sendai S9 peptide (0.5 μg/ml), E/T ratio, 5:1. Values shown are means of triplicate measurements and are representative of the data obtained in three independent experiments.

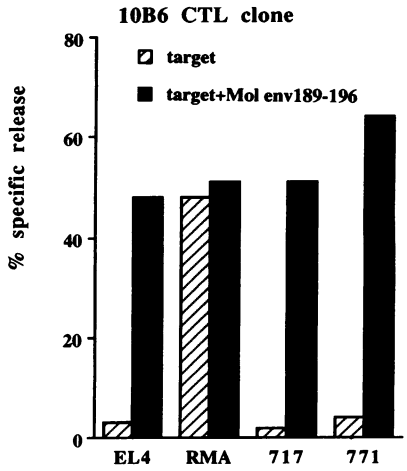


FIG. 5. Lytic activity of the Mol env(189-196)-specific 10B6 CTL clone against MuLV-negative EL4 tumor cells, Rauscher MuLV-transformed RMA tumor cells, and MCF1233 MuLV-transformed 717 and 771 tumor cells without peptide or loaded with Mol env(189-196) peptide (0.5 μg/ml; E/T ratio, 2.5). The values shown are means of triplicate measurements and are representive of the data obtained in three independent experiments. MuLV expression on the various target cells was checked by FACS analysis (data not shown). The MCF1233 MuLV-transformed lymphomas are targets for MCF1233-specific CTL (38).

(total peptide length of 8 aa, with an F or Y at position 5 and an aliphatic residue at position 8 [11]), the 8-mer peptide (aa sequence SSWDFITV) functions optimally in target cell sensitization and probably represents the endogenously processed Moloney MuLV epitope.

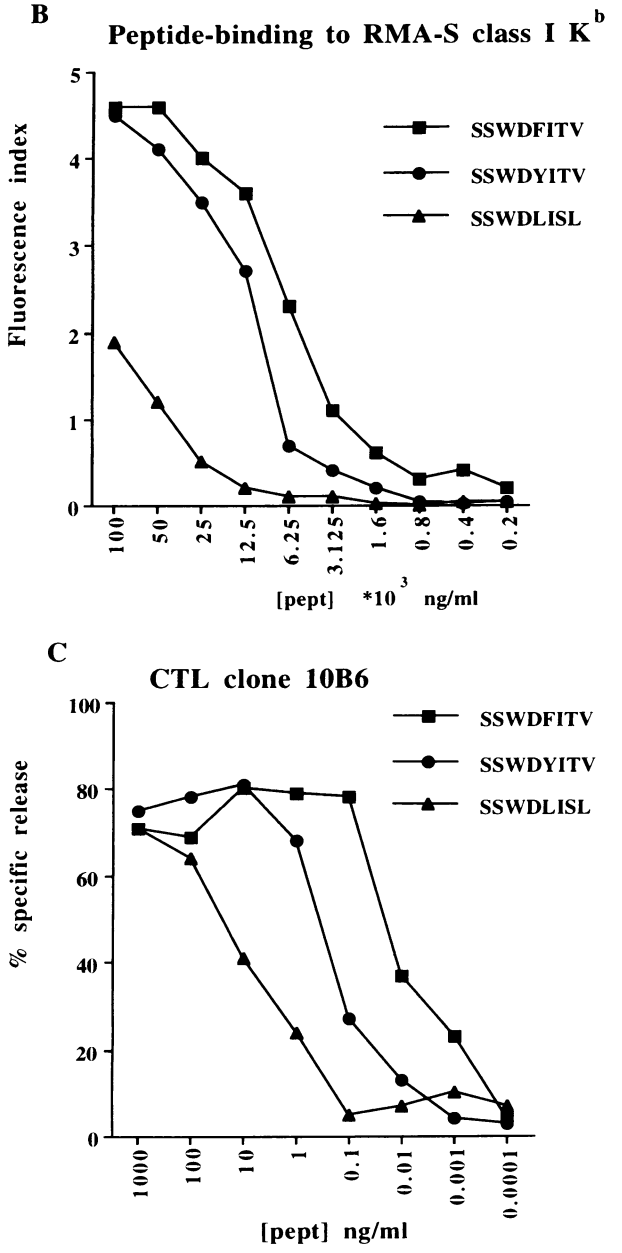
On Moloney MuLV-infected target cells, the env(189-196) epitope is presented by class I K^b but not class I D^b or D^{bm13} (Fig. 1). We were interested to see whether the class I D^{bm13} molecule (of which the antigen-binding pocket is partly shaped by a K^b-derived sequence) could present this peptide when the peptide was supplied at a high concentration. Mouse class I-transfected rat (FRE) target cells were incubated with 0.5 μg of env(189-196) peptide per ml and were tested for recognition by 10B6 CTL. As shown in Fig. 3, only class I K^b-transfected cells and the $H\text{-}2^b$(K^b, D^b) EL4 mouse cell line could be targeted for lysis. Peptide-loaded class I D^{bm13} or D^b transfectants were not recognized, so these molecules seem incapable of presenting the epitope to anti-Moloney MuLV, K^b-restricted CTL.

Recognition of Mol env(189-196) by CTL derived from B6 mice. To investigate whether B6 mice have CTL precursors for the Moloney env(189-196) K^b-presented epitope, peptide immunizations were performed with the synthetic Mol env(189-196) peptide and with the S9 control peptide, encompassing the immunodominant (K^b-restricted) epitope of Sendai virus (19, 33). Mouse spleen cells were restimulated in vitro with peptide-incubated RMA-S cells (which after incubation express the relevant peptide-class I complex at high density [3]); at day 7 they consisted mainly of CD8-positive cells (data not shown). In cytotoxicity experiments, these B6-derived CTL appeared specific for the peptide they were raised against (Fig. 4). Thus, Mol env(189-196)-primed CTL lysed Moloney peptide-loaded target cells, whereas S9-primed CTL were specific for S9-loaded cells. In addition, the Moloney MuLV peptide-specific CTL were capable of lysing RMA tumor cell targets, which endogenously process a Rauscher MuLV-encoded equivalent of the Mol env(189-196) epitope (see below), while S9-specific CTL did not recognize RMA tumor cells. B6 mice therefore have Mol env(189-196)-specific CTL precursors, and these can be activated by appropriate peptide stimulation.

MuLV type specificity of Mol env(189-196)-directed CTL. In $H\text{-}2^b$ mice, MuLV-directed CTL responses generally distinguish between MuLV of the Friend, Moloney, or Rauscher (FMR) type and MuLV of the AKV or MCF type (9, 12, 28). When testing for MuLV specificity, we found the 10B6 CTL clone to recognize the $H\text{-}2^b$ Rauscher MuLV-induced lymphoma RMA (22) but not two $H\text{-}2^b$ MCF1233 MuLV-induced lymphomas, 717 and 771 (37) (Fig. 5). All tumor target cells could be sensitized for recognition by loading with the Mol env(189-196) peptide, which shows their capacity to present this epitope. Figure 6A shows an alignment of the different MuLV sequences in the Moloney epitope encoding region. Both Friend and AKV MuLV encode the sequence SSWDY-ITV, which differs at the position 5 K^b anchor residue from Mol env(189-196) (SSWDFITV) (13, 20). The Rauscher MuLV sequence, obtained by us by PCR analysis of the Rauscher viral transcripts in the Rauscher virus-induced RMA cell line (data not shown), is SSWDFITV, which is identical to the Moloney MuLV sequence. MCF1233 MuLV (a C57BL virus isolate from our laboratory [48]) encodes the sequence SSWDLISL (37a). Corresponding synthetic MuLV peptides were tested in titration experiments for their abilities to upregulate class I K^b expression on RMA-S cells (which correlates with K^b-binding capacity) (Fig. 6B) and for recognition by the 10B6 CTL clone (Fig. 6C). We found that the Moloney/Rauscher-derived peptide was the strongest K^b binder, closely followed by the Friend/AKV-encoded peptide, whereas the MCF peptide (which lacks the position 5 anchor

A

MuLV		aa sequence	ref.
Moloney	env189-196	SSWDFITV	35
Friend	env192-199	SSWD**Y**ITV	20
AKV	env189-196	SSWD**Y**ITV	13
Rauscher		SSWDFITV	DNS
MCF1233	env143-150	SSWD**L**I**S**L	37a



residue) bound poorly to class I K^b. In the CTL tests, all MuLV peptides did equally well at high concentrations (1 μg/ml), but recognition of the MCF peptide tapered off at 100 ng/ml, whereas the Friend/AKV peptide and Moloney/Rauscher peptide could be diluted to concentrations as low as 1 ng/ml and 0.1 ng/ml, respectively. In conclusion, the 10B6 CTL clone, which represents the main specificity of Moloney MuLV-directed CTL in bm13 mice, cross-reacts with RMA tumor cells expressing Rauscher MuLV, showing endogenous processing of the Moloney MuLV epitope equivalent. 10B6 CTL also recognize the Friend and AKV MuLV-encoded epitope equivalent but not the MCF1233-encoded peptide or MCF1233 MuLV-transformed tumor cells.

DISCUSSION

Specific mutations in the antigen binding cleft of major histocompatibility complex molecules alter the array of presented peptides (30, 45) and in this way may influence CTL-mediated immunity. B6 and B6 mutant class I D^{bm13} mice are both capable of eradicating Moloney virus-induced tumors, but in B6 mice the main effectors are class I D^b-restricted CTL, whereas in bm13 mice the CTL response is restricted by the K^b molecule (38–41). In this study, it was shown by clonal analysis that mutant bm13 mice, compared with normal B6 mice, display elevated levels of Moloney MuLV-specific K^b-restricted CTLp. We mapped the immunodominant bm13 CTL epitope of Moloney MuLV from a set of viral peptides that had been selected for class I K^b binding. This method previously had been shown useful for epitope identification (26) and recently enabled us to map a CTL epitope for the MCF1233 MuLV (37).

The Mol env(189-196) epitope identified here fully corresponds to the K^b allele-specific binding motif (5). It possesses a Phe at position 5 (the motif describes a Phe or Tyr) and a Val at position 8 (which is one of the less frequent, carboxy-terminally occurring aliphatic amino acids). In functional assays, the Moloney MuLV env(189-196) peptide did not bind the D^b molecule detectably, nor was it presented to T cells by this molecule. The class I D^{bm13} molecule, containing a K^b-derived sequence in the floor of the antigen-binding cleft (11), also did not present Mol env(189-196) to K^b-restricted CTL.

In B6 mice, although the frequency of Moloney MuLV-directed K^b-restricted CTLp was extremely low following Moloney MSV priming in vivo and Moloney virus restimulation in vitro, Mol env(189-196)-specific CTL could be raised by

FIG. 6. (A) Alignment of the FMR, AKV, and MCF1233 MuLV sequences in the Moloney MuLV env(189-196) epitope-encoding region. Nonconserved amino acid residues are shown in boldface and underlined. The Rauscher sequence was identified by PCR analysis of an mRNA isolate from the RMA cell line (DNS, data not shown). Primers were selected up- and downstream from the epitope, on the basis of the Moloney sequence. This resulted in a 291-bp PCR fragment, which was sequenced (37a). (B) Titration of the Mol env(189-196)/Rauscher peptide and the Friend/AKV and MCF1233 MuLV equivalents in a peptide-binding experiment on RMA-S cells. Binding to class I K^b was measured by FACS analysis. Fluorescence indexes {[(mean fluorescence with peptide) − (mean fluorescence without peptide)]/mean fluorescence without peptide} are depicted on the *y* axis. (C) Titration of the Mol env(189-196)/Rauscher peptide and the Friend/AKV and MCF1233 MuLV equivalents in a cytotoxicity experiment on EL4 target cells. Effector was the 10B6 CTL clone (E/T ratio, 4). The values shown are means of triplicate measurements and are representative of the results obtained in two independent experiments.

immunization with this K^b-presentable peptide. In addition, in CTL bulk cultures of Moloney MuLV-immunized mice, occasionally minor responses against a K^b-presented component are observed (41), and we sometimes detect weak reactivity against Mol env(189-196) peptide-pulsed cells in short-term CTL cultures from B6 tumor regressor mice (our unpublished observations). Together, these facts imply that in B6 mice, the Mol env(189-196) epitope is probably subdominant in the B6 CTL response against Moloney MuLV.

The immunodominance of the env(189-196) epitope in bm13 mice may result simply from failure of the B6 D^b epitope (the identity of which is unknown) to be properly presented by the D^{bm13} molecule, which would allow the subdominant epitope to take over. However, this does not seem the most likely explanation. Our CTLp analyses showed elevated levels for Moloney MuLV-specific K^b-restricted CTLp in bm13 mice, comparable to the levels of D^b-restricted CTLp in B6. Furthermore, it was shown previously that in F_1 (B6 × bm13) offspring mice, inoculation with Moloney virus activates K^b- and D^b-restricted CTL (40), indicating that the presence of class I D^b does not negatively influence a potentially K^b-restricted CTL response. Another B6 derived D^b mutant strain, the bm14 strain (K^b, D^{bm14}), does not mount a detectable CTL response upon inoculation with Moloney virus (CTLp less than 1/100,000 [16, 40]). Bm14 mice carry a single amino acid substitution (position 70, Gln to His) in the α-helix, lining the D^b antigen-presenting groove (the D^{bm14}-specific mutation) (11), but they are otherwise genetically identical to B6 or bm13 mice (which carry the D^{bm13}-specific mutation). The nonresponsiveness of the bm14 haplotype toward Moloney MuLV can be overcome, since upon in vitro restimulation with virus-infected dendritic cells (which are superior in presenting antigen [16]), virus-specific K^b-restricted CTL are detected in bulk spleen cell cultures from infected bm14 mice. We therefore conclude that B6, bm13, and bm14 mice have CTL precursors for a K^b-presented Moloney viral epitope, but the bm13 phenotype favors the positive selection of these CTL.

Because the D^{bm13} molecule has gained sequence homology with the K^b molecule, we hypothesize that in bm13 mice the positive selection of T cells with T-cell receptors (TcR) for class I K^b-presented peptides can take place on the K^b molecule as well as on the D^{bm13} molecule. This would enlarge the precursor population of K^b-restricted CTL. According to the current view, the process of positive selection depends on interaction of the TcR with major histocompatibility complex molecules that are filled with self peptides (1, 15, 24, 34). CTL selected on a particular class I molecule do not necessarily have the ability to recognize their epitope in the context of this molecule. Mice transgenic for the LCMV D^b-presented glycoprotein gp(32-42) epitope TcR positively select this receptor on the D^b molecule as well as on the D^{bm13} molecule, but the D^{bm13} molecule is incapable of presenting the LCMV glycoprotein gp(32-42) epitope to this TcR (25). In our system, we propose that K^b-restricted Mol env(186-196)-specific T cells can be selected on class I D^{bm13}, whereas this class I molecule cannot present env(186-196) to mature CTL. In future studies we will test this hypothesis, by analysis of transgenic mice that carry the Mol env(189-196)-specific TcR.

The second part of this study addressed the fine MuLV specificity of the Moloney virus-directed bm13 CTL response. CTL raised against MuLV in $H\text{-}2^b$ mice usually appear either specific for the exogenous FMR type of MuLV or for the AKV/MCF MuLV type, derived from endogenous mouse MuLV sequences (9, 12, 28, 37). Cross-reactivity is sometimes reported (7). We recently identified an immunodominant MCF1233 MuLV-encoded epitope (MCF M8) (37), presented by class I K^b, which was conserved between AKV and MCF MuLV. The homologous sequence in FMR MuLV differed at the first amino acid position (Lys to Arg). Introduced in the epitope-corresponding synthetic peptide, this single amino acid substitution did not influence the K^b binding but led to drastically reduced recognition by the MCF1233-specific CTLs, thereby explaining their MCF/AKV MuLV type specificity.

The bm13 Mol env(189-196) CTL epitope is identical in Rauscher MuLV, has one amino acid difference (position 5, Phe to Tyr) in Friend and AKV MuLV, and differs at three positions in MCF MuLV (SSWDFITV to SSWDLISL) (Fig. 6A). Rauscher MuLV-transformed cells endogenously process and present the epitope, as is apparent from the specific lysis of RMA tumor cells by the 10B6 CTL clone (Fig. 5) and several other clones tested in this regard (data not shown). RMA recognition by 10B6 CTL is K^b restricted, as was previously shown in a study on viral antigen presentation by the antigen processing defective RMA-S cell line, a derivative of RMA (36). Whether the Mol env(189-196) equivalent is processed and presented by Friend and AKV MuLV-infected cells is not known. Synthetic peptides containing a Tyr for Phe at position 5 (an allowed substitution at an anchor position for K^b binding (5), differed only slightly in binding capacity from the Moloney peptide (Fig. 6B), whereas in CTL assays, 10-fold higher amounts of mutated peptide were required for target cell sensitization (Fig. 6C). MCF1233 MuLV-induced tumors are not recognized by the Mol env(189-196)-specific 10B6 CTL clone. MCF1233 is a recombinant between a milk-transmitted AKV-type MuLV and a C57BL endogenous MCF viral sequence (37a, 48). The epitope equivalent in this virus is located in the MCF-donated region (thus shared by most other MCF MuLV) and contains Leu instead of Phe at position 5, thereby lacking an anchor residue for K^b binding. The corresponding peptide has a low affinity for K^b, as indicated by poor binding in the RMA-S assay and the need for high concentrations in target cell sensitization assays, and hence is unlikely to be presented on MCF-transformed cells.

In conclusion, Moloney MuLV-directed bm13 CTL, specific for the env(189-196) epitope, discriminate between Moloney and Rauscher MuLV on the one hand and MCF MuLV on the other hand. Cross-reactivity toward Friend and AKV MuLV-infected cells remains to be explored. Both this study and the MCF1233 M8 epitope study (37) support the earlier observations on the FMR versus AKV/MCF type specificity of MuLV-directed CTL responses and explain this phenomenon at the single-amino-acid level.

ACKNOWLEDGMENTS

We thank F. Leupe, P. Bijl, and R. van Aalst for biotechnical assistance, J. W. Drijfhout for peptide synthesis, and R. Offringa, F. Koning, and F. Claas for critically reading the manuscript.

This work was supported by the Dutch Cancer Society, grants IKW 89-19 and RUL93-560. M. Kast is a senior fellow of the Royal Netherlands Academy of Arts and Sciences (KNAW).

REFERENCES

1. **Ashton-Rickardt, P. G., L. van Kaer, T. N. M. Schumacher, H. L. Ploegh, and S. Tonegawa.** 1993. Peptide contributes to the specificity of positive selection of $CD8^+$ T cells in the thymus. Cell **73**:1041–1049.
2. **Bjorkman, P. J., M. A. Saper, B. Samraoui, W. S. Bennet, J. L. Strominger, and D. C. Wiley.** 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature (London) **329**:512–518.
3. **de Bruijn, M. L. H., T. N. M. Schumacher, J. D. Nieland, H. L. Ploegh, W. M. Kast, and C. J. M. Melief.** 1991. Peptide loading of

empty major histocompatibility complex molecules on RMA-S cells allows the induction of primary cytotoxic T lymphocyte responses. Eur. J. Immunol. **21**:2963–2970.

4. **de Waal, L. P., W. M. Kast, R. W. Melvold, and C. J. M. Melief.** 1983. Regulation of the cytotoxic T lymphocyte response against Sendai virus analyzed with H-2 mutants. J. Immunol. **130**:1090–1096.
5. **Falk, K., O. Rötschke, S. Stevanovic, G. Jung, and H. G. Rammensee.** 1991. Allele specific motifs revealed by sequencing of self-peptides eluted from MHC-molecules. Nature (London) **351**: 290–296.
6. **Flyer, D. C., S. J. Burakoff, and D. V. Faller.** 1983. Cytotoxic T lymphocyte recognition of transfected cells expressing a cloned retroviral gene. Nature (London) **305**:815–818.
7. **Flyer, D. C., S. J. Burakoff, and D. V. Faller.** 1986. The immune response to Moloney murine leukemia virus-induced tumors: induction of cytolytic T lymphocytes specific for both viral and tumor-associated antigens. J. Immunol. **137**:396–397.
8. **Gausepohl, H., M. Kraft, C. Boulin, and R. W. Frank.** 1990. Automated multiple peptide synthesis with BOP activation, p. 1003. *In* J. E. Rivier and G. R. Marshall (ed.), Proceedings of the 11th American Peptide Symposium. ESCOM, Leiden, The Netherlands.
9. **Gomard, E., J. P. Levy, F. Plata, Y. Henin, V. Duprez, A. Bismuth, and T. Reme.** 1978. Studies on the nature of the cell surface antigen reacting with cytolytic T lymphocytes in murine oncorna-virus-induced tumors. Eur. J. Immunol. **8**:228–236.
10. **Gorer, P. A.** 1950. Studies in antibody response of mice to tumour inoculation. Br. J. Cancer **4**:372–379.
11. **Hemmi, S., J. Geliebter, R. A. Zeff, R. W. Melvold, and S. G. Nathenson.** 1988. Three spontaneous H-2 D^b mutants are generated by genetic micro-recombination (gene conversion) events. Impact on the H-2-restricted immune responsiveness. J. Exp. Med. **168**:2319–2335.
12. **Herberman, R. B., T. Aoki, M. Nunn, D. H. Lavrin, N. Soares, A. Gazdar, H. Holden, and K. S. S. Chang.** 1974. Specificity of ^{51}Cr-release cytotoxicity of lymphocytes immune to murine sarcoma virus. J. Natl. Cancer Inst. **53**:1103–1111.
13. **Herr, W.** 1984. Nucleotide sequence of AKV murine leukemia virus. J. Virol. **49**:471–478.
14. **Holt, C. A., K. Osorio, and F. Lilly.** 1986. Friend virus-specific cytotoxic T lymphocytes recognize both gag and env gene-encoded specificities. J. Exp. Med. **164**:211–226.
15. **Jacobs, H., J. von Boehmer, C. J. M. Melief, and A. Berns.** 1990. Mutations in the major histocompatibility complex class I antigen-presenting groove affect both negative and positive selection of T cells. Eur. J. Immunol. **20**:2333–2337.
16. **Kast, W. M., C. J. P. Boog, B. O. Roep, A. C. Voordouw, and C. J. M. Melief.** 1988. Failure or success in the restoration of virus-specific cytotoxic T lymphocyte response defects by dendritic cells. J. Immunol. **140**:3186–3193.
17. **Kast, W. M., L. P. de Waal, and C. J. M. Melief.** 1984. Thymus dictates major histocompatibility complex (MHC) specificity and immune response gene phenotype of class II MHC-restricted T cells but not of class I MHC-restricted T cells. J. Exp. Med. **160**:1752–1766.
18. **Kast, W. M., and C. J. M. Melief.** 1991. Fine peptide specificity of cytotoxic T lymphocytes directed against adenovirus-induced tumours and peptide-MHC binding. Int. J. Cancer **6**(Suppl.):90–94.
19. **Kast, W. M., L. Roux, J. Curren, H. J. J. Blom, A. C. Voordouw, R. H. Meloen, D. Kolakovsky, and C. J. M. Melief.** 1991. Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. Proc. Natl. Acad. Sci. USA **88**:2283–2287.
20. **Koch, W., G. Hunsmann, and R. Friedrich.** 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. J. Virol. **45**:1–9.
21. **Levy, J. P., and J. C. Leclerc.** 1977. The murine sarcoma virus-induced tumor: exception or general model in tumor-immunology? Adv. Cancer Res. **24**:1–66.
22. **Ljunggren, H. G., C. Öhlen, P. Höglund, L. Franksson, and K. Kärre.** 1991. The RMA-S lymphoma mutant; consequences of a peptide loading defect on immunological recognition and graft rejection. Int. J. Cancer **6**(Suppl.):38–44.
23. **Monaco, J. J.** 1992. A molecular model of MHC class I-restricted antigen processing. Immunol. Today **13**:173–179.
24. **Nikolić-Zugić, J., and M. Bevan.** 1990. Role of self-peptides in positively selecting the T-cell repertoire. Nature (London) **344**:65–67.
25. **Ohashi, P. S., R. M. Zinkernagel, A. Althage, H. Hengartner, and H. Pircher.** 1993. Enhanced positive selection of a transgenic TCR by a restriction element that does not permit negative selection. Int. Immunol. **5**:131–138.
26. **Pamer, E. G., T. Harty, and M. J. Bevan.** 1991. Precise prediction of a dominant class I MHC-restricted epitope of Listeria monocytogenes. Nature (London) **353**:852–855.
27. **Plata, F., E. Gomard, J. C. Leclerc, and J. P. Lévy.** 1973. Further evidence for the involvement of thymus-processed lymphocytes in syngeneic tumor cell cytolysis. J. Immunol. **111**:667–671.
28. **Plata, F., and F. Lilly.** 1979. Viral specificity of H-2-restricted T killer cells directed against syngeneic tumors induced by Gross, Friend, or Rauscher leukemia virus. J. Exp. Med. **150**:1174–1186.
29. **Ramsdell, F., and B. J. Fowlkes.** 1990. Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. Science **248**:1342–1348.
30. **Rohren, E. M., L. R. Pease, H. L. Ploegh, and T. N. M. Schumacher.** 1993. Polymorphisms in pockets of major histocompatibility complex class I molecules influence peptide preference. J. Exp. Med. **177**:1713–1721.
31. **Rötzschke, O., F. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H. G. Rammensee.** 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. Nature (London) **348**:252–254.
32. **Ruan, K.-E., and F. Lilly.** 1991. Identification of an epitope encoded in the env gene of Friend murine leukemia virus recognized by anti-Friend virus cytotoxic T lymphocytes. Virology **181**:91–100.
33. **Schumacher, H. G. M., M. L. H. de Bruijn, L. N. Vernie, W. M. Kast, C. J. M. Melief, J. J. Neefjes, and H. L. Ploegh.** 1991. Peptide selection by MHC class I molecules. Nature (London) **350**:703–706.
34. **Sha, W. C., C. A. Nelson, R. D. Newberry, J. K. Pullen, L. R. Pease, J. H. Russell, and D. Y. Loh.** 1990. Positive selection of transgenic receptor-bearing thymocytes by K^b antigen is altered by K^b mutations that involve peptide binding. Proc. Natl. Acad. Sci. USA **87**:6186–6190.
35. **Shinnick, T. M., R. A. Lerner, and J. C. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
36. **Sijts, A. J. A. M., M. L. H. de Bruijn, J. D. Nieland, W. M. Kast, and C. J. M. Melief.** 1992. Cytotoxic T lymphocytes against the antigen-processing-defective RMA-S tumor cell line. Eur. J. Immunol. **22**:1639–1642.
37. **Sijts, A. J. A. M., F. Ossendorp, E. A. M. Mengedé, P. J. van den Elsen, and C. J. M. Melief.** 1994. An immunodominant mink cell focus-inducing murine leukemia virus encoded CTL epitope, identified by its MHC class I-binding motif, explains MuLV type specificity of MCF-directed CTL. J. Immunol. **152**:106–116.

37a. **Sijts, A. J. A. M., C. J. M. Leupers, E. A. M. Mengedé, W. A. M. Loenen, P. van den Elsen, and C. J. M. Melief.** Cloning of the MCF 1233 murine leukemia virus and identification of sequences involved in viral tropism, oncogenicity and T cell epitope formation. Submitted for publication.

38. **Stukart, M. J., J. Boes, and C. J. M. Melief.** 1984. Recognition of H-2 K^b mutant target cells by Moloney virus-specific cytotoxic T lymphocytes from bm13 (H-2 D^b mutant) mice. I. Full recognition of K^{bm11} by K^b-restricted CTL. J. Immunol. **133**:24–27.
39. **Stukart, M. J., J. Boes, and C. J. M. Melief.** 1984. Recognition of H-2 K^b mutant target cells by Moloney virus-specific cytotoxic T lymphocytes from bm13 (H-2 D^b mutant) mice. II. Relationship of K^{bm3} and K^{bm11} in restriction specificities and allodeterminants. J. Immunol. **133**:28–32.
40. **Stukart, M. J., A. Vos, J. Boer, R. W. Melvold, D. W. Bailey, and C. J. M. Melief.** 1982. A crucial role of the H-2 D locus in the regulation of both the D- and the K-associated cytotoxic T lymphocyte response against Moloney leukemia virus, demon-

strated with two D^b mutants. J. Immunol. **128:**1360–1364.
41. **Stukart, M. J., A. Vos, and C. J. M. Melief.** 1981. Cytotoxic T-cell response against lymphoblasts infected with Moloney (Abelson) murine leukemia virus. Methodological aspects and H-2 requirements. Eur. J. Immunol. **11:**251–257.
42. **Taswell, C., H. R. MacDonald, and J. C. Cerottini.** 1980. Clonal analysis of cytolytic T lymphocyte specificity. I. Phenotypically distinct sets of clones as the cellular basis of cross-reactivity to alloantigens. J. Exp. Med. **151:**1372–1385.
43. **Townsend, A. R. M., J. Rothbard, F. M. Gotch, G. Bahadur, D. Wraith, and A. J. McMichael.** 1986. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell **44:**959–968.
44. **Van Bleek, G. M., and S. G. Nathenson.** 1990. Isolation of an endogenous processed immunodominant viral peptide from the $H\text{-}2K^b$ molecule. Nature (London) **348:**213–216.
45. **Van Bleek, G. M., and S. G. Nathenson.** 1991. The structure of the antigen binding groove of major histocompatibility class I molecules determines specific selection of self-peptides. Proc. Natl. Acad. Sci. USA **88:**11032–11036.
46. **van der Zee, R., W. van Eden, R. H. Meloen, A. Noordzij, and J. D. A. van Embden.** 1989. Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides. Eur. J. Immunol. **19:**43–47.
47. **Vasmel, W. L. E., M. Zijlstra, T. Radaszkiewicz, C. J. M. Leupers, R. E. Y. de Goede, and C. J. M. Melief.** 1988. Major histocompatibility complex class II-regulated immunity to murine leukemia virus protects against early T but not late B-cell lymphomas. J. Virol. **62:**3156–3166.
48. **Zijlstra, M., R. E. Y. de Goede, H. J. Schoenmakers, A. H. Schinkel, W. G. Hesselink, J. L. Portis, and C. J. M. Melief.** 1983. Naturally occurring leukemia viruses in H-2 congenic C57BL mice. III. Characterization of C-type viruses isolated from lymphomas induced by milk transmission of B-ecotropic virus. Virology **125:**47–63.